UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

Department of Food Analysis and Nutrition

Standard Operation Procedure

Authentication of Organic Fruits and Vegetables: Rapid UHPLC-HRMS method for pesticide metabolites screening

1 GOALS

The goal of this document is to describe the generic strategy for authentication of organic fruits and vegetables. The concept described below was demonstrated on authentication of grapes and wine, nevertheless can be employed for any other plant matrix to document pesticides use.

2 THEORETICAL BACKGROUND

Organic crops

European regulation (EC) No 834/2007 on organic production and labelling of organic products states that: Organic production is a system of farm management and food production that combines best environmental practices with a high level of biodiversity, the preservation of natural resources, the application of high animal welfare standards and a production method in line with the preference of certain consumers for products produced using natural substances and processes. European regulation (EC) No 889/2008 laying down rules for the implementation of Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control says that: The use of pesticides, which may have detrimental effects on the environment or, result in the presence of residues in agricultural products, should be significantly restricted, only those (natural) compounds listed at the end of document can be used.

Illegal practices in organic farming: pesticides use

Worth to notice, that illegal use of pesticides represents one of fraudulent practices on organic crops. Modern (synthetic) pesticides rapidly degrade after their application due to both physicochemical factors and through biotransformation (se Figure 1 below). Consequently, pesticide residues in samples might be either undetectable or detected at low concentrations (≤10 µg/kg). In other words, residues control might fail to disclose mislabelling (product from organic farming declared as organic). Under such conditions, a monitoring of pesticide metabolites in samples might be a conceivable solution enabling the documentation of earlier pesticide use. Specifically in situation when residues at 10 µg/kg are found, it might be rather difficult to decide whether their

presence is due to accidental contamination e.g. through atmospheric transport or illegal application. However, as far as in addition to parent pesticides also their metabolites are present, then, it becomes evident that some time ago residues higher than 10 µg/kg were contained thus indicating intentional use.

Strategy to document illegal pesticides use in organic farming

As mentioned in the paragraph above, the analysis of pesticide metabolites in crop labelled as organic may support assessment of the way of its contamination. Analysis of metabolites might pose analytical challenges because pesticide degradation leads to the production of a number of metabolites, differing somewhat in their structure and polarity, moreover they may occur at very low levels due to several metabolic pathways may take place in transformation.

Introduction of demonstration case study

This study was focused on the determination of pesticide residues and their metabolites in samples of grapevine and wine using ultrahigh performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLS-HRMS), with the objective of supporting the possibility of the verification of the method of farming. It documents the identification of pesticide metabolites commonly used in conventional farming and provides a characterization of pesticide degradation during grapevine growth, maturation, and during the wine-making process.

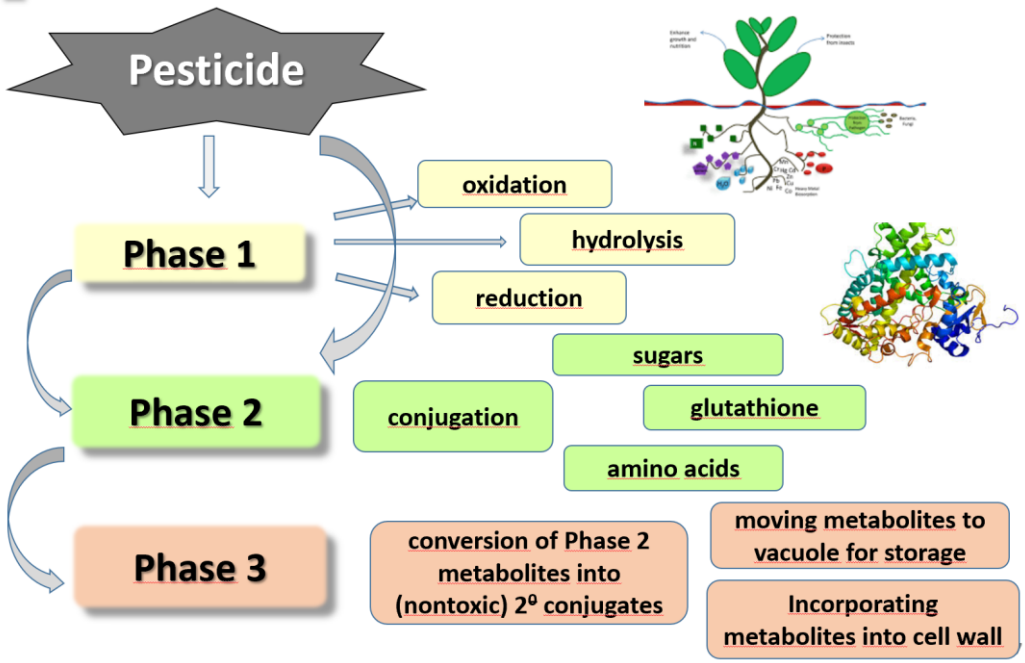

Figure 1 Pesticides biotransformation (pesticide metabolites origination)

3 STANDARD OPERATION PROCEDURE

3.1 MATERIALS

Certified standards of pesticides (dimethomorph, fenhexamid, iprovalicarb, metrafenone, pyraclostrobin, quinoxyfen, spiroxamine, tebuconazole, and triadimenol) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), Honeywell Fluka, or Honeywell Riedel-de Haen (both Seelze, Germany). The purity of standards was in the range of 98–99.9%. The internal standards for triphenyl phosphate (TPP) and nicarbazin were obtained from Sigma-Aldrich (St. Louis, MO). Stock solutions of the individual pesticides were prepared in pure methanol, acetonitrile, or acetone containing 1% formic acid (v/v), depending on the solubility of the specific pesticide. A composite stock standard in acetonitrile was prepared at 50 000 ng mL^{-1} from stock solutions and was stored at −18 °C. The working standard mixtures (20–2000 ng mL^{-1}) used for matrix-matched calibration were prepared from a stock solution by further dilution with acetonitrile.

HPLC-grade acetonitrile, LC–MS-grade formic acid, ammonium formate, and ammonium acetate were obtained from Sigma-Aldrich. Methanol was obtained from Merck (Darmstadt, Germany). Acetone and sodium chloride were obtained from Penta (Chrudim, Czech Republic). Anhydrous magnesium sulfate was obtained from Honeywell Fluka. Deionized water (18 MΩ) was produced using a Millipore Milli-Q system (Bedford, MA).

3.2 SAMPLE PREPARATION

Prior to the analysis, solid samples (vine leaves and wine grapes) were homogenized using a laboratory blender. Liquid samples (musts and wines) were mixed thoroughly. Parent pesticides were determined using an ISO 17025 accredited method routinely used in our laboratory. A new extraction/detection method had to be implemented for the analysis of metabolites.

3.3 EXTRACTION OF PARENT PESTICIDE RESIDUES

The extraction procedure was based on the QuEChERS method. In total, 2.5 g of homogenized vine leaves were weighed into a 50 mL centrifugation tube, followed by

the addition of 10 mL of water containing 1% (v/v) of formic acid. The matrix was allowed to soak for 20 min. In the case of wine grapes/wine, 10 g of previously homogenized samples was weighed into a 50 mL plastic centrifuge tube without water addition. Subsequently, 10 mL of acetonitrile was added and the tube was vigorously shaken for 2 min. In the next step, 1 g of NaCl and 4 g of $MgSO_4$ were added and the shaking process was repeated for 1 min. Then 100 μL of the mixture of TPP and nicarbazin (5 μg mL^{-1}) as an internal standard was added, and the tubes were centrifuged for 5 min at 11 200 rcf. An aliquot of the supernatant was transferred into a vial.

In the case of wine samples, the volume of the extract (top organic layer) was affected by the ethanol naturally present in wines (11–15 vol %). To compensate for this effect, an addition of internal standards was used.

3.4 Identification and Quantification of Pesticide Residues

Identification of pesticide residues in the samples was based on a comparison of retention time, accurate mass (*m*/*z*) of the (de)protonated molecule, isotopic pattern matching, and accurate mass of MS/MS fragments to those obtained for pesticide reference standards. The acceptable mass error of potential elemental composition for the (de)protonated molecule was ±5 ppm. The identification criteria were in accordance with the requirements in the European Commission's guideline SANTE/11813/2017.(1)

Quantification was performed by using a calibration curve based on matrix-matching calibration standards. To obtain matrix-matched standards corresponding to concentration levels 1, 2, 5, 10, 20, 50, and 100 ng mL^{-1}, 50 μL of a specific working standard mixture and 50 μL of internal standard (1 μg mL^{-1}) were added to 900 μL of the blank extract (blank extract diluted with acetonitrile in ratios of 1:9 and 1:99).

3.5 LC–MS Parameters

The LC–HRMS(/MS) analyses of fungicide residues and their metabolites were performed using an Agilent Infinity 1290 LC system (Agilent Technologies), equipped with an Acquity UPLC HSS T3 analytical column (100 mm × 2.1 mm, 1.8 μm particle size, Waters). Mass spectrometry detection was performed using quadrupole-time of

flight mass spectrometry (Agilent Ion-Mobility Q-TOF 6560) in positive and negative electrospray ionization (ESI) modes.

The column temperature was maintained at 40 °C. The injected sample volume was 4 µL. The mobile phases were different for analyses in electrospray positive (ESI+) and negative (ESI−) ionization modes. For compounds detected in the ESI+, mobile phases were (A) water with 5 mM ammonium formate and 0.1% (v/v) formic acid and (B) methanol, respectively. For compounds detected in the ESI–, mobile phases were (A) water with 5 mM ammonium acetate and (B) pure methanol. The gradient was the same in both polarities: the starting mobile phase composition was 5% of the organic phase (B) with a flow of 0.2 mL min^{-1} and linearly changed to 99% (B) with a flow of 0.3 mL min^{-1} in 10 min. This mobile phase composition was held for 2 min simultaneously with the flow rate being changed from 0.3 to 0.4 mL min^{-1}. The column was reconditioned for 2 min in the starting composition of 5% (B) (flow rate, 0.4 mL min^{-1}). The autosampler temperature was maintained at 5 °C.

The MS source conditions were as follows: capillary voltage (VCap) was 4 kV (−4 kV in ESI−); nozzle voltage was 1 kV; gas temperature and sheath gas temperature were 210 and 380 °C, respectively; drying gas flow and sheath gas flow were 10 L min^{-1} and 12 L min^{-1}, respectively, and nebulizer pressure was 342.6 kPa (35 psig) in both acquisition modes. Collision-induced dissociation was performed using nitrogen, and the collision energy was fixed (20 V). Agilent MassHunter Workstation software (version B.07.00; Agilent Technologies) was used for data acquisition and data analysis.

3.6 Strategy for Detection and Identification of Pesticide Metabolites

Detection and identification of pesticide metabolites in sample were based on the calculated accurate mass (*m*/*z*), isotopic pattern matching, and accurate mass of MS/MS fragments. The acceptable mass error of the potential elemental composition for the (de)protonated molecule was ±5 ppm.

At first, high-resolution mass spectra in a full-scan technique (without fragmentation, MS^1) were acquired across the entire chromatographic run, using a mass range of *m*/*z* 100–1100. The obtained data were searched against the database o-f elemental composition of metabolites (csv format of file), created manually based on a survey of

the available literature on pesticide metabolism in plants (see Table 1), and consideration of common metabolic reactions (e.g., oxidation, dealkylation).

Table 1 Library of screened pesticide residues and their metabolites

no.	analyte (parent pesticide and its metabolite)	elemental composition	ref	no.	analyte (parent pesticide and its metabolite)	elemental composition	ref
1	**dimethomorph**	**$C_{21}H_{22}ClNO_4$**		**6**	**quinoxyfen**	**$C_{15}H_8Cl_2FNO$**	
1a	dimethomorph-demethyl	$C_{20}H_{20}ClNO_4$		6a	3-hydroxy-quinoxyfen	$C_{15}H_8Cl_2FNO_2$	(5)
1b	dimethomorph-demethyl glycoside	$C_{26}H_{30}ClNO_9$	(4)	6b	CFBPQ	$C_{15}H_7ClFNO$	
1c	dimethomorph-Z7	$C_{15}H_{13}ClNO_3$		7	**spiroxamine**	**$C_{18}H_{35}NO_2$**	
1d	dimethomorph-Z37	$C_{21}H_{20}ClNO_5$		7a	spiroxamine-*N*-oxide	$C_{18}H_{35}NO_3$	
1e	dimethomorph-hydroxy	$C_{21}H_{22}ClNO_5$		7b	spiroxamine-*N*-desethyl	$C_{16}H_{31}NO_2$	
2	**fenhexamid**	**$C_{14}H_{17}Cl_2NO_2$**		7c	spiroxamine-*N*-despropyl	$C_{15}H_{29}NO_2$	
2a	fenhexamid-glycoside	$C_{20}H_{27}Cl_2NO_7$		7d	spiroxamine-cyclohexanol	$C_{10}H_{20}O$	(6)
2b	fenhexamid-hydroxy	$C_{14}H_{17}Cl_2NO_3$	(3)	7e	spiroxamine-cyclohexanol glycoside	$C_{16}H_{30}O_6$	
2c	fenhexamid-hydroxy glycoside	$C_{20}H_{27}Cl_2NO_8$		7f	spiroxamine-diol	$C_{10}H_{20}O$	
3	**iprovalicarb**	**$C_{18}H_{28}N_2O_3$**		7g	spiroxamine-diol glycoside	$C_{16}H_{30}O_6$	
3a	iprovalicarb-hydroxy	$C_{18}H_{28}N_2O_4$	(7)	8	**tebuconazole**	**$C_{16}H_{22}ClN_3O$**	
3b	iprovalicarb-hydroxy glycoside	$C_{24}H_{38}N_2O_9$		8a	tebuconazole-hydroxy	$C_{16}H_{22}ClN_3O_2$	(8)
4	**metrafenone**	**$C_{19}H_{21}BrO_5$**		8b	tebuconazole-hydroxy glycoside	$C_{22}H_{32}ClN_3O_7$	
4a	metrafenone CL 1500836	$C_{19}H_{20}O_6$		**9**	**triadimenol**	**$C_{14}H_{18}ClN_3O_2$**	
4b	metrafenone CL 3000402	$C_{19}H_{19}BrO_6$	(9)	9a	triadimenol glycoside	$C_{20}H_{28}ClN_3O_7$	
4c	metrafenone CL 379395	$C_{19}H_{19}BrO_6$		9b	triadimenol-hydroxy	$C_{14}H_{18}ClN_3O_3$	(10)
4d	metrafenone CL 197675	$C_{19}H_{19}BrO_7$		9c	triadimenol-hydroxy glycoside	$C_{20}H_{28}ClN_3O_8$	
5	**pyraclostrobin**	**$C_{19}H_{18}ClN_3O_4$**					
5a	pyraclostrobin-hydroxy	$C_{19}H_{18}ClN_3O_5$					
5b	pyraclostrobin-desmethoxy	$C_{18}H_{16}ClN_3O_3$	(11)				
5c	pyraclostrobin-hydroxy glycoside	$C_{25}H_{28}ClN_3O_{10}$					

In the next step, the identity confirmation of metabolites detected in MS^1 was based on data acquired in the MS/MS run. Three categories of fragments were searched: (i) diagnostic ions, known for some groups of fungicides (2); (ii) common fragments

detected in the MS/MS spectrum of the parent pesticide as well as its metabolite; (iii) fragments characterizing a part of molecule with metabolic modification, not detected in the MS/MS spectrum of the parent pesticide.

For identification of the conjugates of the parent pesticide and/or its metabolite, a search for neutral losses (e.g., hexoses) in fragmentation mass spectra was performed.

3.7 METHOD VALIDATION

Performance characteristics (recovery, repeatability, within-laboratory reproducibility, and limit of quantification) were determined for pesticide residues (parent compounds) in vine leaves, grapes, and wine. Validation studies were performed on spiked blank samples. Two spiking levels (0.002 mg kg^{-1} and 0.02 mg kg^{-1} in grapes and wine or 0.008 mg kg^{-1} and 0.08 mg kg^{-1} in vine leaves) were used and analyzed in six replicates. Within-laboratory reproducibility (RSD_R) was determined from ongoing QC-data in routine analyses (Table 2).

As standards of pesticide metabolites were not available, recovery experiments could not be performed. The precision (repeatability) of the method was determined by an analysis of samples containing incurred pesticide metabolites in six replicates (Tab 3).

Table 2 Method validation (n = 6): recoveries (REC), limits of quantification (LOQs), repeatabilities (RSD), reproducibilities (RSD_R), in grapes, wine, and vine leaves

		Grapes					
		0.002 mg kg^{-1}			0.02 mg kg^{-1}		
	LOQ	REC	RSD	RSD_R	REC	RSD	RSD_R
analyte	(mg kg^{-1})	(%)	(%)	(%)	(%)	(%)	(%)
dimethomorph	0.001	93	2	7	92	1	6
fenhexamid	0.001	88	3	10	89	4	5
iprovalicarb	0.001	90	2	13	94	5	8
metrafenone	0.001	84	6	7	97	3	5
pyraclostrobin	0.001	90	2	5	89	1	7
quinoxyfen	0.001	83	4	11	85	2	9
spiroxamine	0.001	94	1	12	90	2	9
tebucionazole	0.001	87	3	9	92	2	5
triadimenol	0.01	<LOQ			92	4	16

Grapes							
		0.002 mg kg^{-1}			0.02 mg kg^{-1}		
	LOQ	REC	RSD	RSD_R	REC	RSD	RSD_R
analyte	(mg kg^{-1})	(%)	(%)	(%)	(%)	(%)	(%)

	Wine						
		0.002 mg kg^{-1}			0.02 mg kg^{-1}		
	LOQ	REC	RSD	RSD_R	REC	RSD	RSD_R
analyte	(mg kg^{-1})	(%)	(%)	(%)	(%)	(%)	(%)
dimethomorph	0.001	96	2	8	91	4	5
fenhexamid	0.001	98	3	9	94	2	5
iprovalicarb	0.001	94	16	11	93	2	9
metrafenone	0.001	94	2	8	95	1	7
pyraclostrobin	0.001	89	2	6	88	1	6
quinoxyfen	0.001	88	3	9	90	1	5
spiroxamine	0.001	91	1	10	84	9	7
tebuconazole	0.001	92	2	11	95	1	5
triadimenol	0.01	<LOQ			96	5	15

	Vine Leaves						
		0.008 mg kg^{-1}			0.08 mg kg^{-1}		
	LOQ	REC	RSD	RSD_R	REC	RSD	RSD_R
analyte	(mg kg^{-1})	(%)	(%)	(%)	(%)	(%)	(%)
dimethomorph	0.004	95	3	9	93	4	8
fenhexamid	0.008	89	3	11	88	2	10
iprovalicarb	0.004	90	11	17	90	2	15
metrafenone	0.004	88	2	9	93	2	9
pyraclostrobin	0.004	89	1	10	88	1	8
quinoxyfen	0.004	81	4	8	82	3	10
spiroxamine	0.004	92	2	12	93	3	10
tebuconazole	0.004	91	2	7	88	2	6
triadimenol	0.04	<LOQ			83	7	18

Table 3 Method validation for pesticide metabolites in vine leaves and grapes: method repeatability (RSD, n = 6)

	vine leaves	grapes
metabolite of pesticide	**RSD (%)**	**RSD (%)**
dimethomorph-demethyl	13	4
fenhexamid glycoside	8	4
fenhexamid-hydroxy	12	3
fenhexamid-hydroxy glycoside	5	2
iprovalicarb-hydroxy	14	8
iprovalicarb-hydroxy glycoside	13	3
metrafenone-CL 1500836	6	-
metrafenone-CL 379395	6	-
metrafenone-CL 3000402	4	-
pyraclostrobin-desmethoxy	6	4
pyraclostrobin-hydroxy	3	6
spiroxamine-*N*-desethyl	14	3
spiroxamine-*N*-despropyl	10	3
spiroxamine-*N*-oxide	2	5
tebuconazole-hydroxy	9	4
tebuconazole-hydroxy glycoside	8	4

4 ILLUSTRATION OF SREENING STRATEGY APPLICATION

The applicability of the described analytical strategy is illustrated through the Figures below. In Figure 2. Fenhexamid and its metabolites detected in grapes after 15 days of treatment with fungicide preparation are shown.

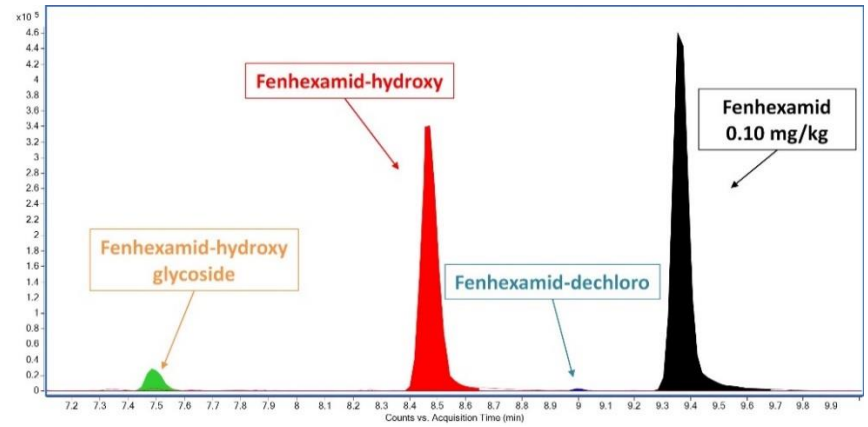

Figure 2. Extracted ion chromatogram (EIC): Fenhexamid (m/z 302.0709) and metabolites Fen-OH (m/z 318.0658) and Fen-dechloro (m/z 268.1099) in grapes.

In Figure 3, the application of the above procedure for screening of penconazole and its metabolites in apples is illustrated (generic approach employed).

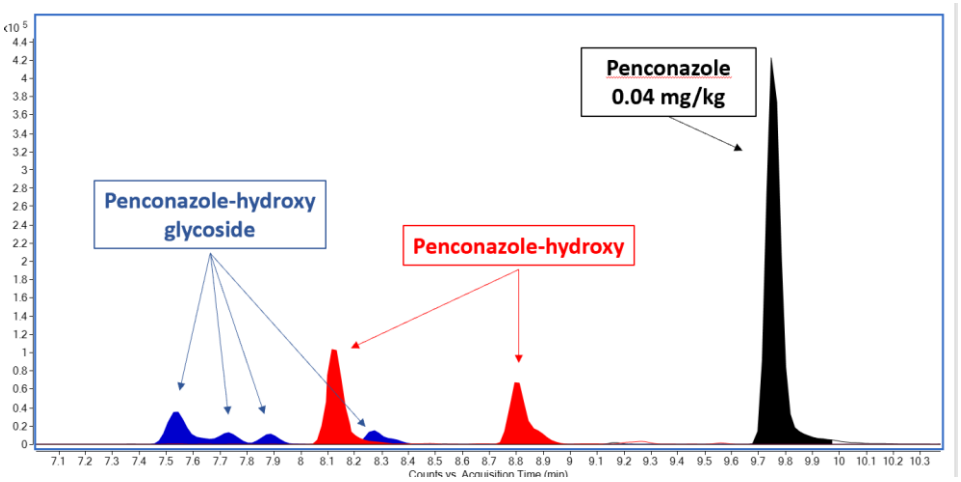

Figure 3. Extracted ion chromatogram (EIC): Penconazole (m/z 284.0721), penconazole-hydroxy (m/z 300.0665) and penconazole-hydroxy glycoside (m/z 462.1176) in apples.

5 APPENDIX

In the attached excel file ´Pesticide metabolites database´ is the overview of potential pesticide metabolites originated from selected parent compounds. These metabolites can be found in various source including JMPR (FAO/WHO) documents EU pesticide database, EFSA etc. Based on their elemental formula respective ions (protonated

deprotonated molecules, their adducts) originated in ESI source can be derived for LC-HRMS.

6 LITERATURE

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7. European Food Safety Authority Conclusion on the peer review of the pesticide risk assessment of the active substance iprovalicarb. *EFSA J.* 2015, *13* (4), 4060, DOI: 10.2903/j.efsa.2015.4060
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10. European Food Safety Authority Conclusion regarding the peer review of the pesticide risk assessment of the active substance triadimenol. *EFSA J.* 2008, *6* (10), 177r, DOI: 10.2903/j.efsa.2008.177r
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PESTICIDES and their METABOLITES

Parent pesticide and their metabolites	Elemental formula
ACETAMIPRID	$C_{10}H_{11}ClN_4$
acetamiprid-desmethyl	$C_9H_9ClN_4$
acetamiprid IM-2-2	$C_9H_{11}ClN_4O$
acetamiprid IM-1-2	$C_{10}H_{14}ClN_4O$
acetamiprid IM-1-3	$C_9H_{11}ClN_2O$
acetamiprid IM-1-4	$C_7H_9N_2Cl$
acetamiprid IM-1-5	$C_9H_{12}N_3Cl$
6-chloronicotinic acid	$C_6H_4ClNO_2$
6-chloronicotinic acid-dehydroxy	C_6H_6ClNO
BOSCALID	$C_{18}H_{12}Cl_2N_2O$
boscalid M510F47	C_6H_4ClNO2
boscalid M510F62	$C_{12}H_{10}ClN$
boscalid-dihydroxy	$C_{18}H_{12}Cl_2N_2O_3$
boscalid-hydroxy	$C_{18}H_{12}Cl_2N_2O_2$
boscalid-hyroxy glycoside	$C_{24}H_{22}Cl_2N_2O_7$
BROMOPROPYLATE	$C_{17}H_{16}Br_2O_3$
4,4'-dibromobenzilic acid	$C_{14}H_{10}Br_2O_3$
BUPIRIMATE	$C_{13}H_{24}N_4O_3S$
ethirimol	$C_{11}H_{19}N_3O$
ethirimol- hydroxy	$C_{11}H_{19}N_3O_2$
de-ethyl-ethirimol	$C_9H_{15}N_3O$
CAPTAN	$C_9H_8Cl_3NO_2S$
THPI	$C_8H_9NO_2$
captan epoxide	$C_8H_7Cl_3NO_3S$
THPI epoxide	$C_{12}H_{15}NO_6$
THPAM	$C_8H_{11}NO_3$
CARBENDAZIM	$C_9H_9N_3O_2$
thiophanate-methyl	$C_{12}H_{14}N_4O_4S_2$
CYPERMETHRIN	$C_{22}H_{19}Cl_2NO_3$
3-phenoxybenzaldehyde	$C_{13}H_{10}O_2$
3-phenoxybenzylalcohol	$C_{13}H_{12}O_2$
3-phenoxybenzoic acid	$C_{13}H_{10}O_3$
3-phenoxybenzylalcohol glycoside	$C_{19}H_{22}O_7$
3-phenoxybenzoic acid glycoside	$C_{19}H_{20}O_{10}$
CYPRODINIL	$C_{14}H_{15}N_3$
cyprodinil-hydroxy	$C_{14}H_{15}N_3O$
cyprodinil-hydroxy glycoside	$C_{20}H_{25}N_3O_6$
1-phenylguanidine	$C_7H_9N_3$
DELTAMETHRIN	$C_{22}H_{19}Br_2NO_3$
3-phenoxybenzylaldehyde	$C_{13}H_{10}O_2$
3-phenoxybenzoic acid	$C_{13}H_{10}O_3$

Parent pesticide and their metabolites	Elemental formula
DIFENOCONAZOLE	$C_{19}H_{17}C_{l2}N_3O_3$
1,2,4-triazole	$C_2H_3N_3$
triazole alanine	$C_5H_8N_4O_2$
triazole acetic acid	$C_4H_8N_4O_2$
difenoconazole CGA 189138	$C_{13}H_8C_{l2}O_3$
difenoconazole CGA 205374	$C_{16}H_{11}C_{l2}N_3O_2$
difenoconazole CGA 205375	$C_{16}H_{14}ClN_3O_2$
difenoconazole-hydroxy	$C_{19}H_{17}Cl2N_3O_4$
difenoconazole-hydroxy CGA 205375	$C_{16}H_{14}ClN_3O_3$
DIMETHOATE	$C_5H_{12}NO_3PS_2$
omethoate	$C_5H_{12}NO_4PS$
dimethoate-hydroxy-glucoside	$C_{11}H_{22}NO_9PS_2$
dimethoate-O-desmethyl	$C_4H_{10}NO_3PS_2$
DIMETHOMORPH	$C_21H_{22}ClNO_4$
dimethomorph-demethyl	$C_{20}H_{20}ClNO_4$
dimethomorph-demethyl glycoside	$C_{26}H_30ClNO_9$
dimethomorph-hydroxy	$C_{21}H_{22}ClNO_5$
DIPHENYLAMINE	$C_{12}H_{11}N$
diphenylamine-glucoside	$C_{18}H_{21}NO_5$
4-hydroxy diphenylamine	$C_{12}H_{11}NO$
DITHIOCARBAMATES	-
aniline	C_6H_7N
maneb	$C_4H_6MnN_2S_4$
mancozeb	$C_8H12_MnN_4S_8Zn$
metiram	$C_4H_6N2S_4Zn$
propineb	$C_5H_8N_2S_4Zn$
thiram	$C_6H_{12}N_2S_4$
ziram	$C_6H_{12}N_2S_4Zn$
ETU (Ethylenethio urea)	$C_3H_6N_2S$
EBDC (ethylenebisdithiocarbamate)	$C_4H_8N_2S_4$
EBIS (ethylenebisisothiocyanate sulfide)	$C_4H_4N_2S_2$
EU (ethylen urea)	$C_4H_{10}N_4S_2$
DITHIANON	$C_{14}H_4N_2O_2S_2$
dithianon-desulfide (D 4110933)	$C_{14}H_4N_2O_2S$
phthalic acid	$C_8H_6O_4$
phthalaldehyde	$C_8H_6O_2$
1,4-naphthoquinone	$C_{10}H_5O_2$
DODINE	$C_{13}H_{29}N_3$
guanidine	CH_5N_3
octylguanidine carboxylic acid	$C_9H_{13}N_3O_2$
hexylguanidine carboxylic acid	$C_7H_{15}N_3O_2$
dodecylguanidine carboxylic acid	$C_{13}H_{17}N_3O_2$

Parent pesticide and their metabolites	Elemental formula
ETHEPHON	$C_2H_6ClO_3P$
HEPA	$C_2H_7O_4P$
ETOFENPROX	$C_{25}H_{28}O_3$
etofenoprox-hydroxy	$C_{25}H_{28}O_4$
alpha-CO (2-(4-ethoxyphenyl)-2-methylpropyl 3 phenoxybenzoate)	$C_{25}H_{26}O_4$
FENAZAQUIN	$C_{20}H_{22}N_2O$
4-OHQ	$C_8H_6N_2O$
TBPE	$C_{12}H_{17}O$
FENPYROXIMATE	$C_{24}H_{27}N_3O_4$
fenpyroximate-demethyl	$C_{23}H_{25}N_3O_4$
fenpyroximate-hydroxy	$C_{24}H_{27}N_3O_5$
fenpyroximate M3	$C_{20}H_{18}N_3O_3$
N-desmethyl Fenpyroximate M3	$C_{19}H_{16}N_3O_3$
FENVALERATE	$C_{25}H_{22}ClNO_3$
CPIA	$C_{11}H_{13}ClO_2$
decarboxy-fenvalerate	$C_{24}H_{22}ClNO$
3-Phenoxybenzoic acid	$C_{13}H_{10}O_3$
FLONICAMID	$C_9H_6F_3N_3O$
TFNA	$C_7H_4NF_3O_2$
TFNG	$C_9H_7N_2F_3O_3$
TFNA-AM	$C_7H_5N_2F_3O$
FLUXAPYROXAD	$C_{18}H_{12}F_5N_3O$
M700F002	$C_5H_4N_2F_2O_2$
M700F008	$C_{17}H_{12}N_3F_5O$
M700F008-glucoside	$C_{23}H_{22}N_3F_5O_6$
FLUIDOXINIL	$C_{12}H_6F_2N_2O_2$
2,2-difluoro-benzo[1,3]dioxole-4-carboxylic	$C_8H_4F_2O_4$
fludioxonil CGA 265378	$C_{12}H_4F_2O_4N$
fludioxonil CGA 308103	$C_8H_6F_2NO_4$
fludioxonil SYN 518579	$C_{12}H_5F_2N_2O_4$
fludioxonil SYN 518580	$C_{12}H_4F_2N_2O_5$
fludioxonil SYN 518581	$C_{12}H_7F_2N_2O_6$
FLUOPYRAM	$C_{16}H_{11}ClF_6N_2O$
fluopyram-hydroxy	$C_{16}H_{11}ClF_6N_2O_2$
fluopyram-hydroxy glycoside	$C_{22}H_{21}ClF_6N_2O_7$
fluopyram-glycoside	$C_{22}H_{21}ClF_6N_2O_5$
fluopyram-benzamide	$C_8H_6F_3NO$
HEXYTHIAZOX	$C_{17}H_{21}ClN_2O_2S$
hexythiazox-hydroxy	$C_{17}H_{21}ClN_2O_3S$
CHLORANTRANILIPROLE	$C_{18}H_{14}BrCl_2N_5O_2$
chlorantraniliprole IN-F6L99	$C_5H_6BrN_3O$
chlorantraniliprole IN-ECD73	$C_{13}H_8Cl_2N_2O$
chlorantraniliprole IN-DBC80	$C_9H_5BrClN_3O_2$
chlorantraniliprole IN-EQW78	$C_{18}H_{12}BrC_{12}N_5O$
chlorantraniliprole IN-F9NO4	$C_{17}H_{12}BrCl_2N_5O_2$
chlorantraniliprole IN-GAZ70	$C_{17}H_{10}BrCl_2N_5O$
chlorantraniliprole IN-H2H20	$C_{18}H_{14}BrCl_2N_5O_3$

Parent pesticide and their metabolites	Elemental formula
CHLORPROPHAM	$C_{10}H_{12}ClNO_2$
3-chloroanilin	C_8H_8ClNO
chlorpropham-hydroxy	$C_{10}H_{12}ClNO_3$
chlorpropham-glukoside	$C_{16}H_{22}ClNO_7$
CHLORPYRIFOS	$C_9H_{11}Cl_3NO_3PS$
TCP (3,5,6-trichloropyridinol)	$C_5H_2Cl_3NO$
chlorpyrifos IIIA	$C_6H_7Cl_3NO_3PS$
chlorpyrifos IIIB	$C_4H_3Cl_3NO_3PS$
DES (desmethyl chlorpyrifos-methyl)	$C_6H_5Cl_3NO_3PS$
chlorpyrifos TMP	$C_6H_4Cl_3NO$
chlorpyrifos-methyl	$C_7H_7Cl_3NPO_3S$
chlorpyrifos-hydroxy	$C_9H_{11}Cl_3NO_4PS$
IMAZALIL	$C_{14}H1_4Cl_2N_2O$
R014821	$C_{11}H_{10}Cl_2N_2O$
INDOXACARB	$C_{22}H_{17}ClF_3N_3O_7$
indoxacarb IN-KB687	$C_9H_8F_3O_3N$
indoxacarb IN-MA573	$C_9H_7ClO_4$
indoxacarb IN-MF014	$C_{10}H_{10}F_3N_3O_4$
indoxacarb IN-MH304	$C_{11}H_9ClO_4$
indoxacarb-hydroxy	$C_{22}H_{17}ClF_3N_3O_8$
IPRODIONE	$C_{13}H_{13}Cl_2N_3O_3$
RP 32596	$C_6H_5Cl_2N$
RP 30228	$C_{13}H_{13}Cl_2N_3O_3$
RP 32490	$C_{10}H_7Cl_2N_3O_3$
RP 35606	$C_{13}H_{14}Cl_2N_3O_3$
RP 30181	$C_6H_{10}N_2O_2$
LAMBDA-CYHALOTHRIN	$C_{23}H_{19}ClF_3NO_3$
lambda-cyhalotrin R157836	$C_{23}H_{19}ClF_3NO_3$
gama-cyhalotrin	$C_{23}H_{19}ClF_3NO_3$
3-phenoxybenzaldehyde	$C_{13}H_{10}O_2$
METHOXYFENOZIDE	$C_{22}H_{28}N_2O_3$
methoxyfenozide-hydroxy	$C_{22}H_{28}N_2O_4$
methoxyfenozide-hydroxy glucoside	$C_{27}H_{38}N_2O_9$
MYCLOBUTANIL	$C_{15}H_{17}ClN_4$
myclobutanil-butyric acid	$C_{13}H_{11}ClN_4O_2$
myclobutanil-oxo	$C_{15}H_{15}ClN_4O$
myclobutanil-hydroxy	$C_{15}H_{17}ClN_4O$
2-PHENYLPHENOL	$C_{12}H_{10}O$
2-methoxybiphenyl	$C_{13}H_{12}O$
2-phenylphenol glycoside	$C_{18}H_{20}O_6$
2-phenylhydroquinon	$C_{12}H_{10}O_2$
PHOSMET	$C_{11}H_{12}NO_4PS_2$
phthalic acid	$C_8H_6O_4$
phthalamic acid	$C_8H_7NO_3$

Parent pesticide and their metabolites	Elemental formula
PIRIMICARB	$C_{11}H_{18}N_4O_2$
pirimicarb-hydroxy	$C_{11}H_{18}N_4O_3$
pirimicarb-desmethyl	$C_{10}H_{16}N_4O_2$
pirimicarb-desmethyl formamid	$C_7H_{11}N_3O$
PROPAMOCARB	$C_9H_{20}N_2O_2$
propamocarb-2-hydroxy	$C_9H_{20}N_2O_3$
oxazolidine	C_3H_7NO
N-desmethyl-propamocarb	$C_8H_{18}N_2O_2$
PROPARGITE	$C_{19}H_{26}O_4S$
TBPC	$C_{16}H_{24}O_2$
TBPC diol	$C_{16}H_{24}O_3$
HOMe TBPC	$C_{16}H_{24}O_3$
HOMe TBPC	$C_{16}H_{24}O_4$
PYRACLOSTROBIN	$C_{19}H_{18}ClN_3O_4$
pyraclostrobin-hydroxy	$C_{19}H_{18}ClN_3O_5$
pyraclostrobin-hydroxy glycoside	$C_{25}H_{28}ClN_3O_{10}$
pyraclostrobin-desmethoxy	$C_{18}H_{16}ClN_3O_3$
PYRIMETHANIL	$C_{12}H_{13}N_3$
pyrimethanil-dihydroxy	$C_{12}H_{13}N_3O_2$
pyrimethanil-dihydroxy glucoside	$C_{18}H_{23}N_3O_7$
pyrimethanil-hydroxy	$C_{12}H_{13}N_3O$
pyrimethanil-hydroxy glucoside	$C_{18}H_{23}N_3O_6$
SPIRODICLOFEN	$C_{21}H_{24}Cl_2O_4$
spirodiclofen-enol	$C_{15}H_{14}Cl_2O_3$
spirodiclofen-enol-hydroxy	$C_{15}H_{14}Cl_2O_4$
2,4-dichloro-mandelic acid glucoside	$C_{14}H_{16}Cl_2O_8$
TEBUCONAZOLE	C16H22ClN3O
tebuconazole glycoside	$C_{22}H_{32}ClN_3O_6$
tebuconazole-hydroxy	$C_{16}H_{22}ClN_3O_2$
tebuconazole-hydroxy glycoside	$C_{22}H_{32}ClN_3O_7$
1,2,4-triazole	$C_2H_3N_3$
triazole acetic acid	$C_4H_5N_3O_2$
TEBUFENOZIDE	$C_{22}H_{28}N_2O_2$
RH-1788	$C_{21}H_{32}N_2O_2$
RH-9886	$C_{22}H_{19}N_2O_3$
RH-2651	$C_{21}H_{29}N_2O_4$
RH-6595	$C_{21}H_{31}N_2O_3$
TEBUFENPYRAD	$C_{18}H_{24}ClN_3O$
tebufenpyrad-demethyl	$C_{17}H_{22}ClN_3O$
tebufenpyrad-hydroxy	$C_{18}H_{24}ClN_3O_2$
tebufenpyrad-dihydroxy	$C_{18}H_{24}ClN_3O_3$
TETRACONAZOLE	$C_{13}H_{11}Cl_2F_4N_3O$
1,2,4-triazole	$C_2H_3N_3$
triazole alanine	$C_5H_8N_4O_2$
triazole acetic acid	$C_4H_8N_4O_2$

Parent pesticide and their metabolites	Elemental formula
THIABENDAZOLE	$C_{10}H_7N_3S$
thiabenzimidazole-hydroxy	$C_{10}H_7N_3SO$
benzimidazole glucoside	$C_{13}H_{16}N_2O_6$
benzimidazole	$C_7H_6N_2$
THIACLOPRID	$C_{10}H_9ClN_4S$
thiacloprid-amid	$C_{10}H_{11}ClN_4SO$
thiacloprid-hydroxy	$C_{10}H_9ClN_4SO$
thiacloprid-sulfoxide	$C_{10}H_9ClN_4S_2O$
6-chloronicotinic acid	$C_6H_4ClNO_2$
TRIFLOXYSTROBIN	$C_{20}H_{19}F_3N_2O_4$

APPLES

Parent pesticide	Elemental formula	Metabolites	Elemental formula
Acetamiprid	$C_{10}H_{11}ClN_4$	acetamiprid-desmethyl	$C_9H_9ClN_4$
		acetamiprid IM-2-2	$C_9H_{11}ClN_4O$
		acetamiprid IM-1-2	$C_{10}H_{14}ClN_4O$
		acetamiprid IM-1-3	$C_9H_{11}ClN_3O$
		acetamiprid IM-1-4	$C_5H_6N_2Cl$
		acetamiprid IM-1-5	$C_6H_{12}N_3Cl$
		6-chloronicotinic acid	$C_6H_4ClNO_2$
		6-chloronicotinic acid-dehydroxy	C_6H_4ClNO
Boscalid	$C_{18}H_{12}Cl_2N_2O$	boscalid M510F47	C_6H_4ClNO2
		boscalid M510F62	$C_{12}H_{10}ClN$
		boscalid-dihydroxy	$C_{18}H_{12}Cl_2N_2O_3$
		boscalid-hydroxy	$C_{18}H_{12}Cl_2N_2O_2$
		boscalid-hyroxy glycoside	$C_{24}H_{22}Cl_2N_2O_7$
Ametoctradin	$C_{15}H_{25}N_5$	Ametoctradin-M650F03	$C_9H_{11}N_4O_3$
		Ametoctradin-M650F04	$C_9H_9N_3O_2$
Difenoconazole	$C_{19}H_{17}Cl_2N_3O_3$	Difenoconazole CGA 189138	$C_{13}H_8Cl_2O_3$
		Difenoconazole CGA 205374	$C_{16}H_{11}Cl_2N_3O_2$
		Difenoconazole CGA 205375	$C_{16}H_{11}Cl_2N_3O_2$
		Difenoconazole-hydroxy	$C_{19}H_{17}Cl_2N_3O_4$
Dimethomorph	$C_{21}H_{22}ClNO_4$	Dimethomorph-Z7	$C_{15}H_{11}ClNO_3$
		Dimethomorph-demethyl	$C_{20}H_{20}ClNO_4$
		Dimethomorph-demethyl glycoside	$C_{26}H_{30}ClNO_9$
		Dimethomorph-hydroxy	$C_{21}H_{22}ClNO_5$
Fenhexamid	$C_{14}H_{17}Cl_2NO_2$	Fenhexamid-glykoside	$C_{20}H_{27}Cl_2NO_7$
		Fenhexamid-hydroxy	$C_{14}H_{17}Cl_2NO_3$
		Fenhexamid-hydroxy glykoside	$C_{20}H_{27}Cl_2NO_8$
		Difenhexamid	$C_{26}H_{32}Cl_4N_2O_4$
Fluxapyroxad	$C_{18}H_{12}F_5N_3O$	M700F002	$C_5H_4N_2F_2O_2$
		M700F008	$C_{17}H_{12}N_3F_2O$
		M700F008-glycoside	$C_{23}H_{22}N_3F_5O_8$
Fluopicolide	$C_{14}H_8Cl_3F_3N_2O$	2,6-dichlorobenzamide	$C_7H_5Cl_2NO$
		3-chloro-5-(trifluoromethyl) pyridine-2-karboxylová kyselina	$C_7H_3ClF_3NO_2$
		Fluopicolide-hydroxy	$C_{14}H_8Cl_3F_3N_2O_2$
Fluopyram	$C_{16}H_{11}ClF_6N_2O$ 3 960 464	Fluopyram-hydroxy	$C_{16}H_{11}ClF_6N_2O_2$
		Fluopyram-hydroxy glycoside	$C_{22}H_{21}ClF_6N_2O$
		Fluopyram-benzamide	$C_8H_6F_3NO$
Chlorantraniliprole	$C_{18}H_{14}BrCl_2N_5O_2$	chlorantraniliprole IN-F6L99	$C_5H_6BrN_3O$
		chlorantraniliprole IN-ECD73	$C_{13}H_8Cl_2N_2O$
		chlorantraniliprole IN-DBC80	$C_8H_5BrClN_3O_2$
		chlorantraniliprole IN-EQW78	$C_{14}H_{12}BrCl_2N_5O$
		chlorantraniliprole IN-F9NO4	$C_{17}H_{12}BrCl_2N_5O_2$
		chlorantraniliprole IN-GAZ70	$C_{17}H_{10}BrCl_2N_5O$
		chlorantraniliprole IN-H2H20	$C_{18}H_{14}BrCl_2N_5O_3$
Imidacloprid	$C_9H_{10}ClN_5O_2$	Imidacloprid-hydroxy	$C_9H_{10}ClN_5O_3$
		Imidacloprid-olefin	$C_9H_8ClN_5O_2$
		Imidacloprid-nitrosamine	$C_9H_{10}ClN_5O$
		Imidacloprid-denitro	$C_9H_{11}ClN_4$
		Imidacloprid-ketone	$C_9H_{10}ClN_5O$
		Imidacloprid-CHMP glycoside	$C_{15}H_{16}ClNO_6$
		Imidacloprid-CHMP gentiobioside	$C_{21}H_{26}ClNO_{11}$
Iprovalicarb	$C_{18}H_{28}N_2O_3$	Iprovalicarb-hydroxy	$C_{18}H_{28}N_2O_4$
		Iprovalicarb-hydroxy glycoside	$C_{24}H_{38}N_2O_9$
Meptyldinocap	$C_{18}H_{24}N_2O_6$	2,4-DNOP	$C_{14}H_{20}N_2O_5$
Metalaxyl	$C_{15}H_{21}NO_4$	Metalaxyl-hydroxy	$C_{15}H_{21}NO_5$
		Metalaxyl-CGA 62826	$C_{14}H_{19}NO_4$
		Metalaxyl-CGA 107955	$C_{13}H_{17}NO_4$
Methoxyfenozide	$C_{22}H_{28}N_2O_3$	Methoxyfenozide-hydroxy	$C_{22}H_{28}N_2O_4$
		Methoxyfenozide-hydroxy glycoside	$C_{28}H_{38}N_2O_9$
		Methoxyfenozide-dihydroxy	$C_{22}H_{28}N_2O_5$
Metrafenone	$C_{19}H_{21}BrO_5$	Metrafenone-CL3000402	$C_{19}H_{19}BrO_6$
		Metrafenone-CL379395	$C_{19}H_{19}BrO_6$
		Metrafenone-CL1500836	$C_{19}H_{20}O_6$
Myclobutanil	$C_{15}H_{17}ClN_4$	Myclobutanil-hydroxy	$C_{15}H_{17}ClN_4O$
		Myclobutanil-hydroxy glycoside	$C_{21}H_{27}ClN_4O_6$
		Myclobutanil-oxo	$C_{15}H_{15}ClN_4O$
		Myclobutanil-butyric acid	$C_{15}H_{15}ClN_4O_2$
Pirimicarb	$C_{11}H_{18}N_4O_2$	pirimicarb-hydroxy	$C_{11}H_{18}N_4O_3$
		pirimicarb-desmethyl	$C_{10}H_{16}N_4O_2$
		pirimicarb-desmethyl formamid	$C_7H_{11}N_3O$
Pyraclostrobin	$C_{19}H_{18}ClN_3O_4$	Pyraclostrobin-desmethoxy	$C_{18}H_{16}ClN_3O_3$
		Pyraclostrobin-hydroxy	$C_{19}H_{18}ClN_3O_5$

		Pyraclostrobin-dihydroxy	$C_{19}H_{14}ClN_3O_6$
		Pyraclostrobin-hydroxy glycoside	$C_{25}H_{24}ClN_3O_{10}$
		Pyraclostrobin-konjugát	$C_{24}H_{26}ClN_3O_9$
		Pyraclostrobin 500M55	$C_{25}H_{27}ClN_2O_{10}$
Pyrimethanil	$C_{12}H_{13}N_3$	Pyrimethanil-hydroxy	$C_{12}H_{13}N_3O$
		Pyrimethanil-hydroxy glycoside	$C_{18}H_{23}N_3O_6$
		Pyrimethanil-dihydroxy	$C_{12}H_{13}N_3O_2$
		Pyrimethanil-dihydroxy glycoside	$C_{18}H_{23}N_3O_7$
Spirotetramat	C21H27NO5	Spirotetramat metabolite:BYI08330 enol-glucoside	C24H33NO8
		Spirotetramat metabolite:BYI08330-enol	C18H23NO3
		Spirotetramat metabolite:BYI08330-ketohydroxy	C18H23NO4
		Spirotetramat metabolite:BYI08330-monohydroxy	C18H25NO3
Spiroxamine	C18H35NO2	Spiroxamine-N-oxid	$C_{18}H_{31}NO_3$
		Spiroxamine-N-desethyl	$C_{16}H_{31}NO_2$
		Spiroxamine-N-despropyl	$C_{15}H_{29}NO_2$
		Spiroxamine-kyselina	$C_{18}H_{31}NO_3$
		Spiroxamine-cyclohexanol	$C_{18}H_{35}O$
		Spiroxamine-cyclohexanol glycoside	$C_{24}H_{45}O_6$
		Spiroxamine-diol	$C_{18}H_{35}O_2$
		Spiroxamine-diol glycoside	$C_{24}H_{45}O_7$
Tebuconazole	$C_{16}H_{22}ClN_3O$	Tebuconazole-hydroxy	$C_{16}H_{22}ClN_3O_2$
		Tebuconazole-hydroxy glycoside	$C_{22}H_{32}ClN_3O_7$
		Tebuconazole-glycoside	$C_{22}H_{32}ClN_3O_6$
Tebufenozide	$C_{22}H_{28}N_2O_2$	Tebufenozide-hydroxy	$C_{22}H_{28}N_2O_3$
		Tebufenozide-oxo	$C_{22}H_{26}N_2O_3$
		Tebufenozide-aldehyde	$C_{22}H_{26}N_2O_3$
Thiacloprid	$C_{10}H_9ClN_4S$	thiacloprid-amid	$C_{10}H_{11}ClN_4SO$
		thiacloprid-hydroxy	$C_{10}H_9ClN_4SO$
		thiacloprid-sulfoxide	$C_{10}H_9ClN_4S_2O$
		6-chloronicotinic acid	$C_6H_4ClNO_2$